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TITLE: The Effects of Diesel Exhaust and Stress on the Acute Phase Response and Symptoms in the Chemically Intolerant

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13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*
Purpose: The proposed study is designed to test a model of Gulf War Illness, in which simultaneous acute exposures to DE and psychological stress cause increased symptoms via the acute phase response (APR), among individuals reporting chemical intolerance (CI). Scope/Approach: In this double-blind, 2 (DE exposure) X 2 (high vs. low CI) X 2 (stress vs. no stress) design, 110 healthy men and women, ages 21 to 45, will be exposed, during two one-hour exposure sessions, to DE standardized to 300 $\mu g/m^3$ PM_{10} and to ambient air masked with a one-minute pulse of DE. Symptoms, end-tidal CO_2, markers of the APR in peripheral blood and markers of inflammation in the lungs and nasal mucosa will be assessed at baseline and up to 24 hours post exposure. Results and Major Findings: The diesel exhaust exposure system has been constructed and CO, NO_x, $PM_{2.5}$, PM_{10}, BC, volatile fraction, and particle distributions generated in the CEF have all been measured. Further tests are being conducted to improve the delivery method in order to attain a quick and stable target concentration of PM_{10}.

Significance: The controlled exposure system is generating consistent concentrations of DE. Subject exposures can begin when the DOD IRB has approved the study protocol for testing of human subjects.

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Introduction

Exposures to diesel exhaust (DE) and other petrochemical combustion products were the exposures reported by the greatest percentage of all Gulf War veterans (GWV). Along with diesel exhaust and other chemical exposures, psychological stress has been implicated in the onset of unexplained symptoms such as chemical sensitivity among GWV. The purpose of the proposed study is to test a model for chemical sensitivity in GWV, in which simultaneous acute exposures to DE and psychological stress cause increased symptoms via the acute phase response (APR), in susceptible individuals. Individuals who are low or high in the susceptibility factor of chemical intolerance (CI) will be exposed to DE either with or without a public speaking task, an acute psychological stressor. In this double-blind, 2 (DE exposure) X 2 (high vs. low CI) X 2 (stress vs. no stress) design, 110 healthy men and women, ages 21 to 45, will be exposed, during two one-hour exposure sessions, to DE standardized to 300 $\mu g/m^3$ PM_{10} and to clean air masked with a one-minute pulse of DE. Symptoms, end-tidal CO_2, markers of the APR in peripheral blood and markers of inflammation in the lungs and nasal mucosa will be assessed at baseline and up to 24 hours post exposure.

Body

Goals and Objectives for Year 1:

I. August, 2003 – February, 2004

A. Exposure procedures and protocol development: The diesel exhaust system was designed and installed during this six month period. Pilot exposure concentrations have been conducted and exposure monitoring protocols developed. Details of the system are outlined below.

1. **Details of the Diesel Exhaust System:** The diesel engine is a single piston Yanmar YDG5500E (Yanmar Inc. Japan). The delivery line is a system of pipe with flanges, t-connectors, elbows, valves and bypasses made up of 4-inch diameter stainless steel pipe. All elbows and t-connections are also 4-inch diameter stainless steel pipe and have been welded into position. The control valves are connected in-line by flanges. A snug fitting, custom-built flexible stainless steel pipe connects to the exhaust pipe from the generator and adapts, via a flange, to the 4-inch stainless steel delivery pipe. This flexible pipe is not welded to the engine, but is tight enough to be leak proof as determined by odor and spot check monitoring with a real-time SidePak (TSI Inc., St. Paul, MN) particle monitor and a Langan T15.v CO data logger (Langan Products Inc., San Francisco, CA).

Down-stream from the engine, the delivery line comes to the first t-connection. The perpendicular pipe from this connection is vertical and up in orientation. This short length of pipe has a wheel operated control valve that remains fully open for the experiments (as determined by experiment). Immediately following the control valve is an elbow connector that leads to a NPT (pipe thread) t-connection manifold which houses the 6-inch diameter exhaust pipe. The exhaust pipe is about 40 feet in length, oriented vertically, and protruding approximately 12 feet above Environmental and Occupational Health Sciences' (EOHSI) roof. The exhaust line is equipped with a rain guard to keep moisture from infiltrating the system. A secondary valve is equipped at the base of the exhaust line, and can be opened to allow condensate to drain out of the system. This valve remains closed while the engine is in operation and is opened at least one hour after the experiments are complete. This allows enough time for any water vapor to cool, condense, and accumulate at the base of the exhaust line. When this valve is opened, the condensed water drains into a bucket.

From the first t-connector described above, the delivered diesel exhaust passes through the horizontal pipe to the second control valve. This valve controls how much diesel exhaust is delivered to the controlled environment facility (CEF). This valve is also wheel controlled, and is fully opened when the wheel is turned 8.75 full revolutions in the counter-clockwise direction from fully closed. From the closed position, it has been experimentally determined that 7.75 full revolutions in the counterclockwise direction allows the appropriate flow of diesel exhaust to pass through the delivery line in order to produce 300 $\mu g/m^3$ PM_{10} in the CEF. The delivered gas passes through this valve to a second t-connector. The horizontal orientation from this t-connector is capped with a

welded flange (note: This flange is currently capped, but will be used in the future for other projects). The perpendicular pipe from the t-connector is horizontally oriented in the down direction. This delivery pipe passes through the floor of the penthouse and runs within the walls of EOHSI to the third floor ceiling where the clean air delivery plenum to the CEF is located. The final connection to the delivery plenum of the CEF is made using Teflon wrapped pipe thread.

Refer to Figure 1 for a schematic of the system described above.

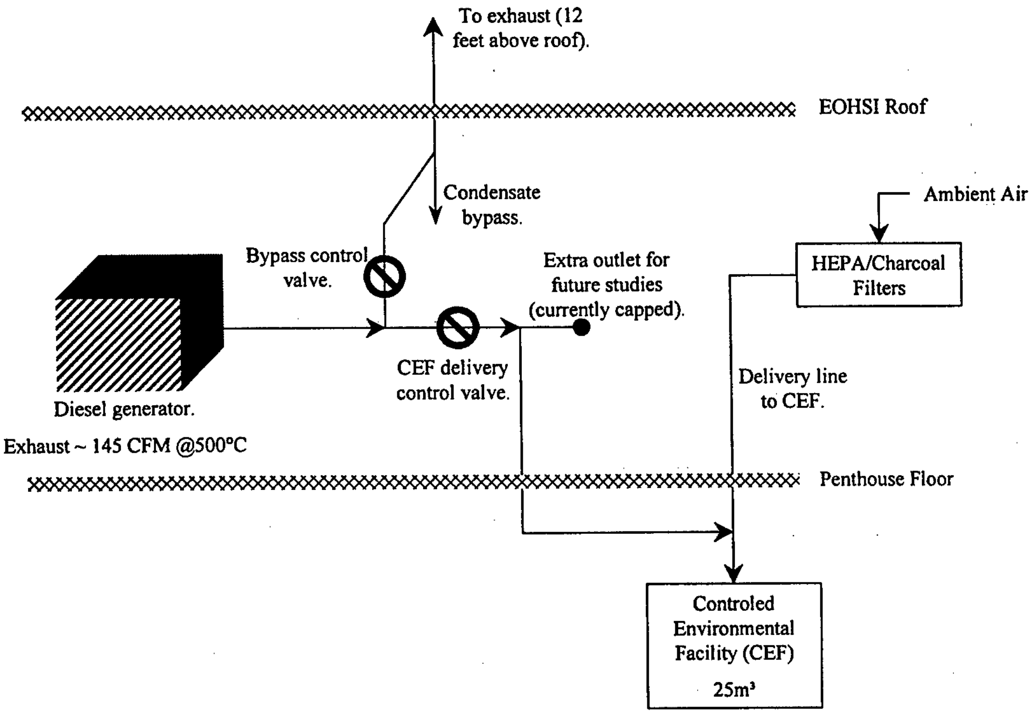

Figure 1. Schematic of the diesel exhaust system delivering diesel exhaust particles (DEP) diluted with fresh air to the Controlled Environmental Facility (CEF).

2. CEF Conditions: The nominal conditions of the CEF were set as follows for all of the tests that delivered diesel exhaust to the CEF. The through flow of air was set to 300 cubic feet per minute, resulting in an air exchange rate of about 1/3 per minute or 20 per hour. The temperature was 72°F. The relative humidity was 35 – 45%. The CEF is designed to hold a negative pressure inside in order to prevent leaks out of the controlled environment.

3. Leak Tests: During the leak tests, the first (bypass) control valve was fully opened and the second control valve was fully closed. This arrangement forced all of the diesel exhaust out of the exhaust line through the roof of EOHSI. A Langan T15.v real-time

CO data-logger with display was present during the leak tests for personal protection. The leak tests were performed at all welded and non-welded points of the diesel exhaust system.

In the Penthouse where the engine is located, leak tests were conducted using the Side-Pak real-time particle monitor in survey mode. The inlet was equipped with a $PM_{2.5}$ impactor. The concentration of $PM_{2.5}$ monitored in the Penthouse before the engine was started was 20 – 30 μg/m³. After the engine was turned on, all connection points along the exhaust pipe were spot monitored using the SidePak. All welded points passed the leak check with $PM_{2.5}$ concentrations within the background range. A leak was observed at the flange connection from the flexible pipe connected directly to the generator exhaust. The leak was observed by a 10 times increase in particle mass concentration measured around the flange. Tightening the flange bolts was sufficient to remediate the leak. A follow-up leak check of the flange measured $PM_{2.5}$ within the background range.

The connections in the EOHSI 3rd floor ceiling (above the CEF) were tested for leaks in the same manner as above, using both, a model 3007 Condensation Particle Counter (CPC) (TSI Inc., St. Paul, MN) capable of surveying ultra-fine particles (diameter > 0.01 μm) by number concentration, and the SidePak. Both monitors surveyed background particle concentrations around all of the connections. A solution of soap water was also used to look for any leaks at these connections. There were no observable leaks by the bubble method.

4. Establishing 300 μg/m³ PM_{10} in the CEF: A test was performed to establish the appropriate degree of opening the bypass and CEF delivery control valves in order to maintain 300 μg/m³ PM_{10} inside the CEF. The Side-Pak monitor was equipped with a PM_{10} impaction inlet for this test. The Side-Pak was located in the control room outside of the CEF. The continuous air sample was collected through a length of Teflon® tubing connected to the CEF through a bulkhead. The background PM_{10} concentration was established at the lower limit of the instrument (3 μg/m³). Two technicians conducted this experiment. One technician was located in the Penthouse to operate the engine and control valves. The second technician was located in the control room of the CEF to monitor the real-time PM_{10} concentration. The two technicians communicated via shortwave radios.

Four space heaters were connected to the generator in order to operate the engine under the full load condition. The CEF delivery control valve was fully closed and the exhaust bypass was fully open to start the experiment. The engine was turned on and warmed up for 5 minutes. The experiment commenced by opening the CEF delivery control valve incrementally until the desired concentration was achieved. The results of the experiment are illustrated in Figure 2. The appropriate degree of opening the control valves was determined to be 7.75 turns and fully open for the CEF delivery control and the bypass valves, respectively.

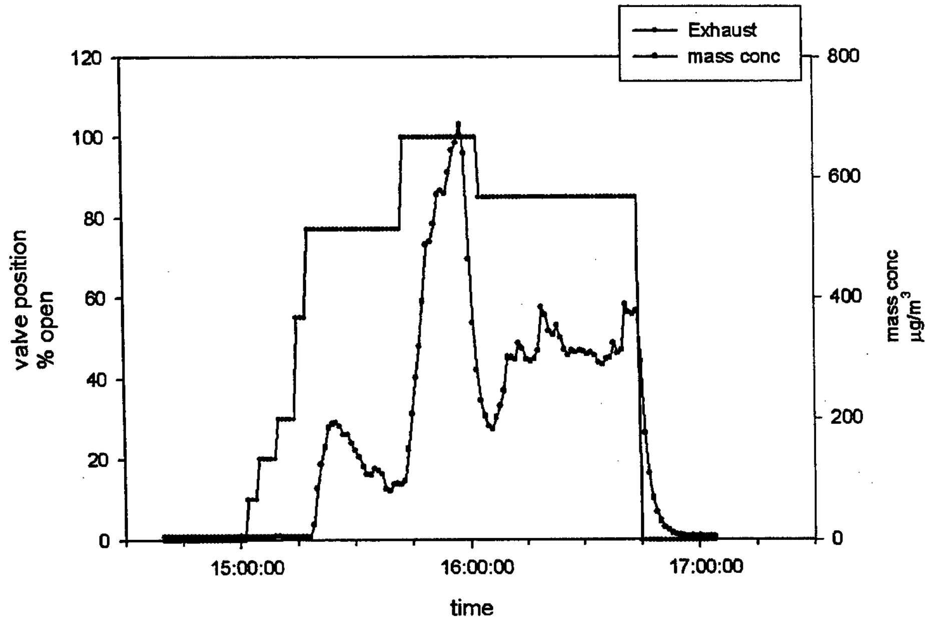

Figure 2. PM_{10} mass concentrations correlating to varying degrees of opening the CEF delivery control valve when the exhaust control valve is fully open. The necessary degree of opening is about 88.5% (7.75 out of a possible 8.75 turns).

5. The Effect of Engine Load on Several DEP Components: NO_x, $PM_{2.5}$, black carbon, and volatile component were measured while varying the load on the engine. The instruments used for this experiment were a model 42C NO_x monitor (Thermo Electron Corp., Franklin, MA), TEOM 1400 (R&P Inc., Albany, NY), MAAP model 5012 (Thermo Electron Corp., Franklin, MA), and a FDMS 8500 volatile (R&P Inc., Albany, NY) for NO_x, $PM_{2.5}$, black carbon, and volatile component, respectively. Engine load was adjusted using four variable output, 1500 Watt, space heaters connected to the generator. The tested conditions were: 1) Engine at idle; 2) 25% load; 3) 50% load; 4) 75% load; and 5) 100% (full) load. Load was adjusted by adding space heaters operated at their full capacity. One space heater was used for the 25% condition, two for 50%, three for 75%, and four space heaters were used for the 100%, full load conditions. Under idle conditions, all of the four space heaters were turned off. This condition resulted in maximizing the $PM_{2.5}$ and volatile components of the delivered diesel exhaust, whereas NO_x and black carbon were minimized. As load increased, NO_x and black carbon concentrations also increased while $PM_{2.5}$ and volatile components decreased. Under full load, the concentrations in the CEF during the experiment were: NO_x = 3.6 ppm, $PM_{2.5}$ = 145 µg/m³, black carbon = 24µg/m³, and volatile component = 15 µg/m³ (See Figure 3 below).

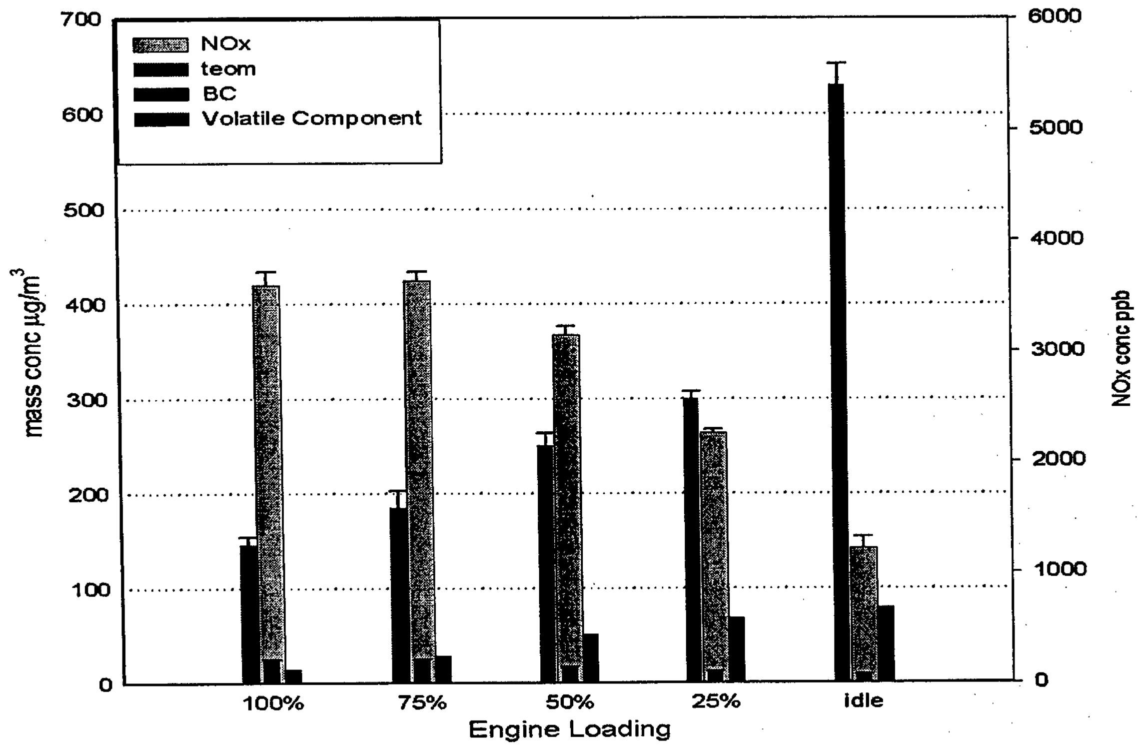

Figure 3. DEP component variability correlating to engine load. Under full load, the DEP components generated in the CEF were NO_x = 3.6 ppm, $PM_{2.5}$ = 145 µg/m³, black carbon = 24µg/m³, and volatile component = 15 µg/m³.

6. DEP Size Distribution: A model 1002 LASAIR (Particle Measuring Systems Inc., Boulder, CO) real-time particle distribution counter and model 3007 CPC were used to measure size distributions of the particles delivered to the CEF under full engine load conditions. Assuming that a density of 1 g/cm³ can characterize the ultrafine particles, a theoretical mass concentration was determined for each of the particle size fractions. The LASAIR measures total particle numbers distributed in eight particle size fractions. The particle size distributions are 0.1 – 0.2, 0.2 – 0.3, 0.3 – 0.4, 0.4 – 0.5, 0.5 – 0.7, 0.7 – 1.0, 1.0 – 2.0, and >2.0 µm. The CPC measures the total number of particles with a diameter greater than 0.01 µm. The composition of the smallest size fraction of particles was determined by subtracting the data collected from the LASAIR from the data collected from the CPC. The smallest reported particle diameter is 0.01 µm, but actually is composed of particles from 0.01 – 0.1 µm. The representative size, known as the geometric mean, was determined by $\sqrt{0.01\mu m \times 0.1\mu m}$. The data indicate that ultrafine particles compose the majority of the DEP generated. The greatest number of particles measured represents those with the 0.01-0.1 µm aerodynamic diameters, whereas the greatest mass of DEP was composed of particles with an aerodynamic diameter of 0.1-0.2 µm. The particle distribution data are illustrated in Figure 4 below.

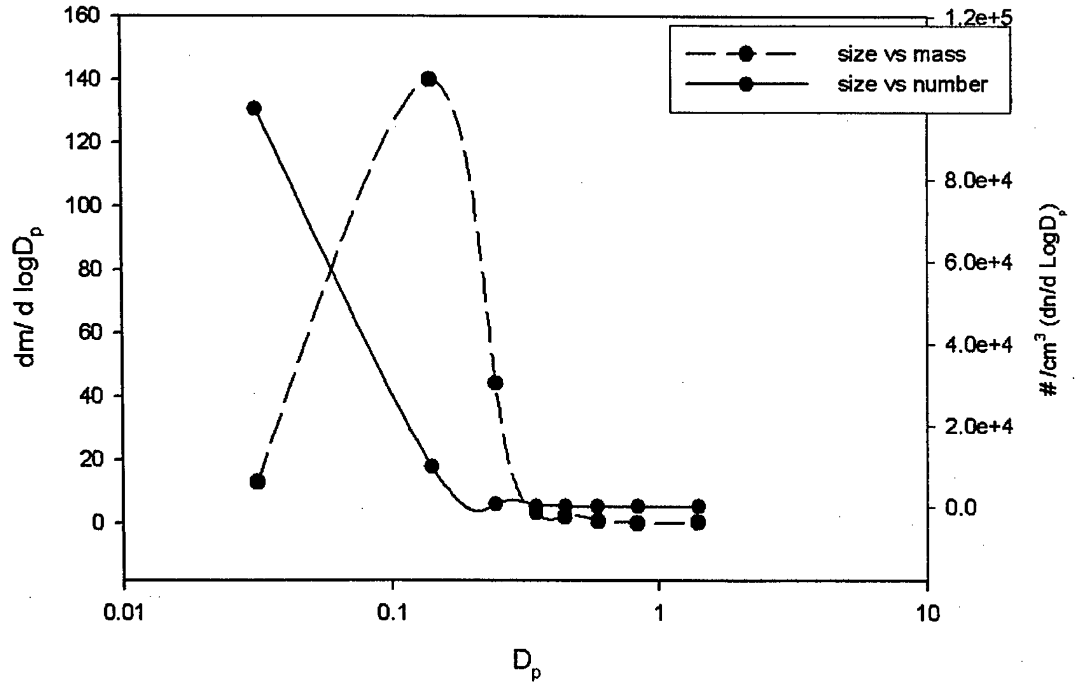

Figure 4. Particle number and theoretical mass concentration versus aerodynamic diameter distributions from data collected using a LASAIR particle distribution counter and a CPC ultrafine particle counter.

7. Carbon Monoxide: A model T15.v CO data logger (Langan Products Inc., San Francisco, CA) was used to monitor carbon monoxide concentration under the desired conditions. Typically the data logger has a resolution of 55 ppb, but this unit has been modified, as per Langan Products Inc. specifications, to achieve a resolution of 5 ppb. The data logger uses an electrochemical sensor to passively measure CO levels in the ambient air. The monitor was placed in the CEF while a PM_{10} concentration of 300 $\mu g/m^3$ was generated. The engine was operated at full capacity with the CEF delivery control valve open to 7.75 revolutions from the closed position. The real-time data was recorded at one minute averaging intervals. The collected data illustrated in Figure 5 below is smoothed on a five minute rolling average. The average CO concentration at the stable condition (from 120 – 162 minutes) was 4.403 ppm. This is well below the OSHA TWA and NIOSH ceiling standards of 50 and 200 ppm, respectively (Note: The ACGIH TWA is 25 ppm).

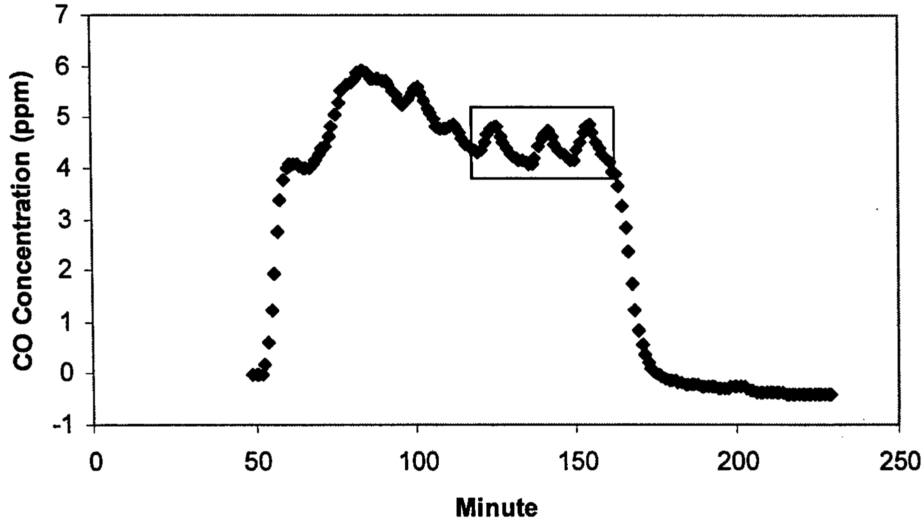

Figure 5. Carbon monoxide concentration inside the CEF during an experiment operating the generator at 100% capacity. The average CO concentration of 4.403 ppm is determined from the data under the stable condition, as represented by the points shown within the box.

7. Noise: It was observed during the initial experimentation period that the delivery system produces a very loud noise through the delivery pipes. This noise is loudest within the CEF. The noise is an amplification of the noise from the engine's exhaust pipe echoing through the delivery pipes. A custom built muffler is on order and will be installed between the engine and the first t-connection. Once the muffler is in place, the particle size distribution will be re-examined.

8. Characterization: CO, NO_x, $PM_{2.5}$, PM_{10}, BC, volatile fraction, and particle distributions generated in the CEF have all been measured and reported here using real-time data-logging instrumentation. More characterization experiments are continuously on-going. Aldehydes, PAH's, and gravimetric PM_{10} with EC content are being measured using non-realtime (integrated) methods. Further tests are being conducted to improve the delivery method in order to attain a quick and stable target concentration of PM_{10}.

9. Subject Involvement: The final testing of the delivery system and characterization measurements is on-going and will be re-evaluated after the custom muffler is installed in the delivery system. It is unlikely that the incorporation of the muffler will result in any significant differences in DEP characterization. Subjects' involvement can begin following the installation of the muffler and completion of the subsequent re-characterization of diesel exhaust delivered into the CEF.

B. **Subject Recruitment:** Subject screening questionnaires are prepared and ready to be administered. However, we have been unable to begin the recruitment process because we have not yet received IRB approval from the Department of Defense. Materials for the University of Medicine and Dentistry of New Jersey IRB were submitted and approved in May of 2003. The protocol was submitted to the DOD IRB in August of 2003 and has been awaiting review. The initial review took place in June of 2004 when

the DOD committee decided to defer the protocol until further information was obtained. The committee questioned the relevance of diesel exhaust exposure for Gulf War Illness and expressed concerns about the health risks of diesel exhaust exposure. The IRB is requesting information from RADIII regarding the relevance of the proposal and from Army experts who have an understanding of diesel health effects. We have not received any information in writing about the status of our review. In response to the verbal report from our project liaison, Dr. Ferrandino, we have prepared a document outlining the relevance of diesel exhaust for Gulf War Illness and reviewing other studies using this exposure in human subjects. We are currently awaiting further information in writing from the DOD IRB. We cannot proceed with any subject recruitment until these issues are resolved.

II. March, 2004 – July, 2004

A. Complete exposure sessions for 30 subjects: See I. B.
B. Complete data coding and entry for 30 subjects: See I. B.
C. Recruit 50 subjects for physical examination screening to participate in Year 2 exposure sessions: See I. B.
D. Prepare annual report for the U.S. Army Medical Research and Materiel Command Volunteer Registry Database: See I. B.

Key Research Accomplishments

None to date.

Reportable Outcomes

Our center has submitted a grant proposal for a human diesel exhaust exposure study to NIEHS/EPA in response to an RFA for studies of mechanisms of cardiovascular effects of air pollution (Howard Kipen, PI). This proposal has been reviewed, received a fundable score, and will be funded by the EPA. The diesel exhaust exposure concentration in this proposed study is the same as in the DOD-funded study (300 µg/m3 PM2.5) with a longer duration (2 hours). The studies share some common endpoints, such as measures of pulmonary inflammation in induced sputum, but the EPA study focuses on more immediate systemic responses including platelet activation and endothelial dysfunction. This award will make use of the diesel exhaust system and pilot work made possible by the award of DOD funds.

Conclusions

We have the capability of conducting controlled exposure to DE with our system. Efficient review by the DOD IRB would allow us to meet the Statement of Work objectives in a timely manner. Our protocol has been deferred as of August, 1, 2004. Therefore, we must wait before we can conduct pilot work with human subjects. This is continuing to significantly delay our project.